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(54) Title: INTERLEUKIN-2 STIMULATED T LYMPHOCYTE CELL DEATH FOR THE TREATMENT OF AUTOIMMUNE DISEASES, ALLERGIC DISORDERS, AND GRAFT REJECTION

(57) Abstract

A method for the treatment or prevention of autoimmune diseases, allergic or atopic disorders, and graft rejection is provided, comprising inducing the death by apoptosis of a subpopulation of T lymphocytes that is capable of causing such diseases, while leaving substantially unaffected the majority of other T lymphocytes. Cell death is achieved by cycle(s) comprising challenging via immunization these T cells with antigenic substance at short time intervals, or by immunization followed by administering interleukin-2 (IL-2) when these T cells are expressing high levels of IL-2 receptor so as to cause these T cells to undergo apoptosis upon re-immunization with the antigenic peptide or protein. These methods are applicable to the treatment of autoimmune diseases such as, for example, multiple sclerosis, uveitis, arthritis, Type I insulin-dependent diabetes, Hashimoto's thyroiditis, Grave's thyroiditis, autoimmune myocarditis, etc., allergic disorders such as hay fever, extrinsic asthma, or insect bite and sting allergies, food and drug allergies, as well as for the treatment or prevention of graft rejection.

INTERLEUKIN-2 STIMULATED T LYMPHOCYTE CELL DEATH FOR THE TREATMENT OF AUTOIMMUNE DISEASES, ALLERGIC DISORDERS, AND GRAFT·REJECTION

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to the treatment and prevention of diseases that are primarily due to T cell immune responses. In particular, it relates to the suppression or elimination of certain autoimmune diseases, graft rejection, and allergic disorders by treatment with interleukin-2 (IL-2) and the specific antigen involved, thus allowing the killing of the subpopulation of T cells that recognizes this specific antigen. In this manner, IL-2 pretreatment sensitizes T cells to undergo programmed cell death following T cell receptor engagement.

Description of Related Art

Stimulation of the $\alpha\beta$ antigen receptor of mature T lymphocytes can lead to either proliferation or programmed cell death (1-4). Programmed cell death, termed apoptosis, is one mechanism for the clonal deletion of both thymocytes and mature T cells that establishes tolerance (5-9). A minor population (approximately 5%) of T lymphocytes of unknown function, termed $\gamma\delta$ cells, has been shown to undergo apoptosis following IL-2 treatment and antigenic stimulation (28). The role of apoptosis in the normal immune response, and the mechanism by which a mature T cell selects between proliferation and death, were not previously understood.

SUMMARY OF THE INVENTION

The present invention arose from the discovery that IL-2 programs mature T cells for antigen-driven death. The T cell death caused by IL-2 followed by antigen stimulation has the hallmarks, such as DNA fragmentation and sensitivity to cyclosporin A, of "programmed cell death" or apoptosis. Thus, IL-2 acts as a death cytokine that will cause the demise only of T cells that are specifically stimulated through their

antigen receptor. This novel use of a previously undiscovered
property of IL-2 will allow the specific elimination of
certain classes of antigen receptor-bearing T cells, forming
the basis for new clinical applications of IL-2.

5   A determinant of the choice between T lymphocyte
proliferation or programmed cell death is the prior exposure
of these cells to interleukin-2 (IL-2). Antigen receptor
stimulation in T cells not exposed to IL-2 causes normal
activation, leading to IL-2 production and growth. In
10 contrast, both CD4$^+$ and CD8$^+$ cells previously exposed to IL-2
undergo apoptosis after antigen receptor stimulation.
Therefore, antigen-activated T cells that are under the
influence of IL-2 will respond to rechallenge by antigen by
undergoing apoptosis. The timing is significant because later
15 antigenic stimulation after the cells are no longer under the
influence of IL-2 will cause growth rather than apoptosis.
Antibody blockage of IL-2 but not IL-4 reverses the rapid and
drastic reduction of lymph node Vβ8$^+$ cells caused in mice by
the bacterial superantigen Staphylococcus bureus enterotoxin
20 B. Thus, IL-2 may participate in a feedback regulatory
mechanism by predisposing mature T lymphocytes to apoptosis.

               At least three uses for IL-2 are made possible based
on this discovery. First, there is an emerging set of
findings that show that infusion of peptides derived from
25 antigens involved in autoimmune diseases leads to a reduction
in severity of such diseases (cf. 73). A variety of studies
of the autoimmune disease experimental allergic encephalitis
(EAE) shows that it is caused by the activation of T cells
upon immunization with myelin basic protein (MBP).
30 Interestingly, infusion of peptides derived from the MBP
sequence that stimulate the T cells that generate the disease
are effective at blocking the disease (60). The discovery
disclosed herein provides an explanation for these seemingly
paradoxical observations, which is that the T cells are
35 activated and stimulated by IL-2 during peptide infusion, and
then undergo apoptosis when they are restimulated by the MBP
antigen. Human diseases that have been associated with T cell
activation by peptide antigens include multiple sclerosis and

autoimmune uveitis (67; 69; 107). It is envisioned that these
diseases, and, for example, systemic lupus erythematosus,
systemic vasculitis, polymyositisdermatomyositis, systemic
sclerosis (scleroderma), Sjogren's Syndrome, ankylosing
5   spondylitis and related spondyloarthropathies, rheumatic
fever, hypersensitivity pneumonitis, allergic bronchopulmonary
aspergillosis, inorganic dust pneumoconioses, sarcoidosis,
autoimmune hemolytic anemia, immunological platelet disorders,
cryopathies such as cryofibrinogenemia, autoimmune
10   polyendocrinopathies, and myasthenia gravis can be approached
by therapy which can now be modulated in a rationale way using
IL-2 and the relevant peptide to cause apoptosis of the T
cells responsible for the disease. The appropriate timing of
IL-2 infusion or a repetitive immunization schedule could
15   substantially augment the protective effect of the infused
peptides.

        Secondly, there is a significant body of literature
that suggests that pre-immunization of an animal or man prior
to engraftment with a foreign tissue prolongs the survival
20   time of the graft (cf. 108). One example of this phenomenon
is the "donor transfusion effect," in which transfusing a
patient about to receive an organ transplant with blood from
the organ donor decreases rejection of the transplant. It is
shown herein that CD8 cells are quite susceptible to
25   IL-2-mediated apoptosis, and this is the primary class of T
cells involved in graft rejection. Based on the discovery of
this novel property of IL-2, CD8+ T cells may be induced to
undergo IL-2-mediated apoptosis; administering IL-2 during and
immediately after the preimmunization/transfusion phase, or
30   repetitive immunization with MHC antigen at appropriately
short intervals, could augment T cell death, leading to
greater tolerance of grafts.

        Thirdly, a wide variety of atopic or allergic
disorders, commonly known as asthma or allergies, results from
35   the effects of activating T cells, which causes both the
release of harmful lymphokines and the production of IgE by B
cells (100, 101). Over the past few decades, clinicians have
made primitive attempts to treat these diseases by a

"desensitization" process consisting of repetitive exposure to the same antigen that elicited the allergy (102). Despite the fact that very little is known about the mechanisms set in play by this procedure, in some cases such treatments were

5   highly successful (102). An important scientific by-product of this work in clinical allergy is that considerable effort has gone into identifying proteins and other molecules that cause allergic responses (100). This has led to the identification of protein sequences for antigens such as Amb a

10   V and Amb t V, which are ragweed allergens that cause hay fever, the protein sequence and characterization of antigenic peptides from allergen M that causes allergy to codfish (105), and the molecular cloning of the cDNA for antigen 5 of white-face hornet venom, associated with allergy to hornet

15   stings (103). Drugs that can cause allergy are typically small organic molecules that may become immunogenic by forming covalent complexes with host proteins. In addition, a large variety of allergens have been prepared as protein extracts to be administered clinically to humans under the supervision of

20   the Food and Drug Administration, and evaluated by a Panel on Review of Allergenic Extracts (102). With the molecular identification of these and other allergy-evoking antigens, it will be possible to immunize in cycle with IL-2 to induce apoptosis of T cells involved in allergic disorders such as

25   allergic rhinitis, bronchial asthma, anaphylactic syndrome, urticaria, angioedema, atopic dermatitis, allergic contact dermatitis, erythema nodosum, erythema multiforme, Stevens-Johnson Syndrome, cutaneous necrotizing venulitis, and bullous skin diseases.

30   The key feature of each of these treatment protocols is that only the antigen-specific T cells which are a small component of the patient's T cell repertoire would be eliminated. The treatment would leave the patient's immune system largely intact. This is in contrast to present

35   treatments that rely upon general immunosuppression that seriously incapacitates the host's immune function (see 109). Moreover, because this treatment causes death of the T lymphocytes, it is superior to other recently discovered

mechanisms which do not kill T cells but rather cause
functional inactivation or anergy which is typically
reversible (98, 99). The experimental results described below
therefore have broad clinical significance in applications to
5 human immunological diseases.

        Throughout the history of immunological approaches
to human and animal diseases, beginning with the first
vaccination against smallpox carried out by Edward Jenner in
1798, the emphasis has been on stimulating a positive and
10 protective antigen-specific immune response. In modern
immunology, this is known to be due to activating lymphocytes.
Hence, causing the activation and proliferation of
antigen-specific immune cells, especially T lymphocytes, forms
the basis of most of the clinical applications of immunology.
15 In particular, the recent advent of molecularly cloned
cytokines, especially those with the ability to cause the
proliferation of immune cells, has furthered the clinical
application of immunology. Such molecularly cloned cytokines
can be readily prepared pharmacologically, and are powerful
20 agents for stimulating the growth and division of lymphocytes.
The conceptual and practical advance offered by the discovery
disclosed herein is that cytokines such as IL-2, when given in
sufficient quantity, also stimulate negative regulatory
effects such as T cell apoptosis. These regulatory effects
25 represent built-in mechanisms to limit or suppress the immune
response. Thus, the recognition that these mechanisms exist,
and the identification of a biologic, IL-2, that potently
evokes antigen-specific T cell death, offers the opportunity
to exploit the negative regulation of the immune response for
30 the treatment of disease.

        Accordingly, it is an object of the present
invention to provide a method for treating or preventing a
disease in a human or animal caused by antigen-activated T
cells, comprising inducing the death by apoptosis of a
35 subpopulation of T lymphocytes that is capable of causing said
disease to an extent greater than that of other T lymphocytes.
Said disease can include an autoimmune disease, graft
rejection, or an allergic or atopic disorder, and said

apoptosis can be achieved by exploiting endogenous IL2, or by
administering this substance exogenously. When IL-2 is
administered exogenously, apoptosis can be achieved by a cycle
comprising challenging via immunization said T cells with a

5   substance selected from the group consisting of an antigen, a
peptide, a protein, a polysaccharide, an organic molecule, and
a nucleic acid, followed by administering a high dose of IL-2
when said T cells are expressing high levels of IL-2 receptor,
so as to cause said T cells to undergo apoptosis upon

10   reimmunization with said substance. When endogenous IL-2 is
employed to achieve apoptosis, said cycle comprises
challenging via immunization said T cells by repeated
administration of said substance at intervals appropriate to
cause apoptosis without the subsequent administration of a

15   high dose of IL-2, relying instead on endogenous levels of
IL-2.

            Further scope of the applicability of the present
invention will become apparent from the detailed description
and drawings provided below. However, it should be understood

20   that the detailed description and specific examples, while
indicating preferred embodiments of the invention, are given
by way of illustration only, since various changes and
modifications within the spirit and scope of the invention
will become apparent to those skilled in the art from this

25   detailed description.

                    BRIEF DESCRIPTION OF THE DRAWINGS
            The above and other objects, features, and
advantages of the present invention will be better understood

30   from the following detailed descriptions taken in conjunction
with the accompanying drawings, all of which are given by way
of illustration only, and are not limitative of the present
invention, in which:
            Fig. 1 shows apoptosis resulting from antigen or

35   anti-CDε stimulation of A.E7 T cells after IL-2 pretreatment.
(A) Photomicrographs of A.E7 T cells pretreated with 100 units
of IL-2, then stimulated and stained with trypan blue. (Top
panels) Representative fields of A.E7 T cells (small round

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cells) and DCEK APCs (large fibroblastic cells) either with no
antigen (left) or 1 μM pigeon cytochrome C peptide antigen
(right). In addition to T cell death, antigen activation of
A.E7 cells leads to the production of factors that cause lysis
5   of DCEK APCS. (Bottom panels) Representative fields of A.E7 T
cells in either untreated wells (lower left) or wells pre-
coated with 10 μg/ml anti-CD3ε (lower right). Some
non-adherent dead T cells and cell fragments, but not live
cells, are washed away during the staining. Dark cells are
10   cells that have died. (B) DNA prepared from equivalent
numbers of A.E7 T cells was subjected to agarose gel
electrophoresis and ethidium bromide staining. Lanes are from
cells treated as in (A) with IL-2 and/or anti-CDε (145-2Cl1)
as indicated. End lanes (M) contain pBR322/Msp I DNA markers.
15   Fig. 2. shows IL-2 dependent clonal elimination of
Vβ8 T cells by immunization with SEB. Histograms of flow
cytometry analysis of lymph node T cells taken from mice that
were either uninjected (Control), injected with SEB (SEB) ,
injected with SEB and the MAb 3C7 that blocks the binding of
20   IL-2 to the IL-2 receptor α chain (SEB + anti-IL-2R), or
injected with SEB and the MAb 11b11 that blocks IL-4 (SEB +
anti-IL4).
                Fig. 3 shows the decrease in human T cell number
when given IL-2 followed by stimulation through the T cell
25   receptor CD3 polypeptide using the monoclonal antibody OKT3.
                Fig. 4 summarizes the therapeutic protocol for the
induction of apoptosis of the present invention.
                Fig. 5 shows the time course of expression of the
IL-2 receptor on human peripheral blood T cells after
30   stimulation with various antigens.
                Fig. 6 shows results of experiments in which the
non-transformed, CD4+ T_H1 T lymphocyte clone A-E7 was
stimulated with increasing concentrations of its cognate
peptide -- pigeon cytochrome c amino acids 81-104.
35   Figs. 7A-7C are photomicrographs showing death of T
lymphocytes treated at high antigen doses.
                Fig. 8 shows decreased T cell number quantitatively
accounts for the suppression of ^{3}HTdR cpm using a FACS

viability assay.   Panel a. is a representative experiment in which CD4+ cells are analyzed for the presence of Vα11 T cell receptor.

Fig. 9 shows that endogenous production of IL-2 is necessary and sufficient for antigen specific T cell death as determined using the FACS viability assay.

Fig. 10 shows the protocol for induction and treatment of EAE.

Fig. 11 shows the results from experiments using repetitive injections of MBP and IL-2 to prevent EAE.

Fig. 12 shows that repetitive injections of 400μg MBP can prevent progression of early stage EAE.

Fig. 13 shows that repetitive injections of 400μg MBP blocks relapses of EAE, thus demonstrating the effectiveness of this therapy to ameliorate existing disease.

Fig. 14 shows FACS results demonstrating that repetitive doses of MBP delete DiI stained cells.

Fig. 15 presents the results of experiments showing that repetitive doses of MBP antigen delete MBP transgenic lymphocytes in vivo.

Fig. 16 shows the protocol for therapy of EAU.

Fig. 17 shows the results of therpy of EAU as measured by scoring of EAU by ocular histopathology.

Fig. 18 shows the schedule of immunization and treatment in the production of serum IgE in response to chicken egg albumin.

Fig. 19 shows the measurement of serum IgE to determine the degree of allergic response in mice.

Fig. 20 shows flow cytometry analysis of human T cells isolated from a patient diagnosed with multiple sclerosis that are specifically reactive with myelin basic protein which were deleted using the present invention.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The disclosures of each of the references cited in the present application are herein incorporated by reference in their entirety.

The following detailed description of the invention is provided to aid those skilled in the art in practicing the

same. Even so, the following detailed description should not
be construed to unduly limit the present invention, as
modifications and variations in the embodiments herein
discussed may be made by those of ordinary skill in the art
5   without departing from the spirit or scope of the present
inventive discovery.

Therapeutic Applications in Human and Veterinary Medicine

10   I.   General Principles
The discovery that interleukin-2 (IL-2) predisposes
T lymphocytes to programmed cell death, or apoptosis, allows
for a novel method of therapeutic intervention in disease
processes in humans and animals primarily caused by the action
15   of T cells (30). In essence, this involves specifically
inducing the death of a subpopulation of T lymphocytes that
are capable of causing disease, while leaving the majority of
T lymphocytes substantially unaffected. This method of
intervention contrasts with, and is potentially far superior
20   to, currently used therapeutic methods that cause a general
suppression or death of T lymphocytes. Examples of
widely-used general immunosuppressive agents are
corticosteroids, such as preunisone, which are used to treat
autoimmune diseases and allergic conditions, and cyclosporin
25   A, which is used for treating graft rejection (31). These
treatments suffer from the drawback of severely compromising
immune defenses, leaving the patient vulnerable to infectious
diseases. The two key elements of the present process are
that: i) only the subset of T cells that reacts with antigens
30   that cause the disease are affected by the treatment; and ii)
the T cells affected by the treatment are killed, i.e., they
are permanently removed from the repertoire.
Several general principles underlie the present
process. T cells recognize antigen in the form of short
35   peptides that form noncovalent complexes with major histo
compatibility complex (MHC) proteins on the surface of
antigen-presenting cells found throughout the body (32).
Antigens may also take the form of polysaccharides, organic

molecules, or nucleic acids. Each T cell bears a unique
receptor called the T cell receptor (TCR) that is capable of
recognizing a specific antigen-MHC complex. Through
rearrangement of the gene segments containing the
5   protein-coding segments of the TCR, a vast array, perhaps a
virtually unlimited number of combinations, of different TCRs
are generated (33). By a mechanism termed "allelic
exclusion", each T cell bears a single unique TCR. The T cell
repertoire is therefore a large number of T cells, each with a
10   distinct TCR that recognizes a specific antigen-MHC complex.
It is this vast array of T cells that allows immunological
responses to the diversity of antigenic structures on invading
microorganisms, tumor cells, and allografts, thus preserving
the integrity of the organism.

15   Most antigens are able to elicit a response in only
a very tiny fraction of the T cell repertoire (34). For
example, the initial response to protein antigens may involve
as few as 1/1000 to 1/10,000 T lymphocytes (35). For this
reason, diseases caused by T cell reactivity are mediated by
20   only a small subset of the large repertoire of T cells (36).
In particular, in those cases where it has been directly
measured, such as in multiple sclerosis, the fraction of the T
cell repertoire which mediates disease is quite small (36).
The important feature of the T cell subset that participates
25   in disease is that it involves T cells which specifically
recognize an antigen that provokes the disease. In allergic
conditions, the antigen causes the release of inflammatory
response molecules. In autoimmune diseases, the antigen may
be derived from a specific organ in the body and, when
30   recognized by a subset of T cells, stimulates the T cells to
attack that organ. A similar effect occurs during graft
rejection. Antigenic proteins in the transplanted organ evoke
a response in a subset of T cells that attacks the engrafted
tissue. For unknown reasons, the fraction of T cells
35   recognizing foreign or "allo" tissue is significantly higher
than the number that will typically recognize a protein
antigen. Nonetheless, the number of responding T cells is

still a distinct minority (1-10%) of the overall T cell
repertoire (37).

In a typical response to a specific antigen-MHC
complex, the T cells undergo a cascade of gene activation
5   events that results from stimulation of the TCR (38). These
have been extensively characterized at the molecular level,
and two such activation events are especially germane to the
present therapy: production of the lymphokine IL-2, and,
expression of the cell surface proteins that constitute high-
10   affinity receptors for IL-2. IL-2 is a 15,000 dalton protein
that causes T cells bearing the appropriate high affinity
receptor to divide (68). Non-activated T cells do not express
high affinity IL-2 receptors (39). The production of IL-2
followed by its interaction with its receptor causes an
15   autocrine mechanism that drives the T cells into the cell
cycle (39). This leads to an initial expansion of T cells
that are specifically reactive with the antigen. The present
inventive discovery indicates that IL-2 also has the
surprising effect of predisposing the expanded pool of either
20   human or mouse T cells to apoptosis or programmed cell death
if they are again stimulated or rechallenged through the TCR
(30, 74). In the present work described supra, the degree of
apoptosis achieved in either human or mouse T cells is
correlated positively with both the level of IL-2 the cells
25   experience during their initial expansion, the strength of the
TCR stimulation upon rechallenge, and the timing of the
rechallenge. The effects of IL-2 wear off 2-3 days after IL-2
is no longer present, hence rechallenge must occur within that
period (94). The process of activation and apoptosis
30   eventually depletes the antigen-reactive subset of the T cell
repertoire. Apoptosis denotes a type of programmed cell death
in which the T cell nucleus shrinks, the genetic material
(DNA) progressively degrades, and the cell collapses (1, 40).
Evidence would suggest that cells cannot recover from
35   apoptosis, and that it results in irreversible killing (1,
40). T cells that do not undergo apoptosis but which have
become activated will carry out their "effector" functions by
causing cytolysis, or by secreting lymphokines that cause B

cell responses or other immune effects (41). These "effector"
functions are the cause of tissue damage in autoimmune and
allergic diseases or graft rejection. A powerful approach to
avoiding disease would therefore be to permanently eliminate
5   by apoptosis only those T cells reactive with the
disease-inciting antigens, while leaving the majority of the T
cell repertoire intact.

        By using IL-2 as an agent that predisposes T cells
to death by TCR stimulation in appropriate cycle with
10   immunization with the antigen(s) leading to autoimmune disease
or graft rejection, the death of disease-causing T cells can
be invoked. Specific methods are described for i) treatment
of autoimmune or allergic diseases by identified protein
antigen and IL-2, and ii) treatment of graft rejection by
15   blood cell antigens and IL-2. Such methods, by logical
extension, can be further developed for other diseases of man
or animals that result from the effects of T cells activated
by specific antigens. Because the vast majority of immune
responses depend on T cell activation, whether cytotoxic
20   responses or antibody production are involved, it is predicted
that this form of therapy could be applied to a wide variety
of autoimmune and allergic conditions (100, 106).

II.   <u>Method for IL-2/peptide-mediated apoptosis of T</u>
25   <u>lymphocytes.</u>

        In several human autoimmune diseases, data have
indicated that antigen-activated T cells play a key role in
the production of disease. These include but are not limited
to: 1) multiple sclerosis (42-47); 2)uveitis (48, 49)) ; 3)
30   arthritis (50-52) ; 4) Type I (insulin-dependent) diabetes
(53, 54); 5) Hashimoto's and Grave' s thyroiditis (55-57) ;
and 6) autoimmune myocartiditis (58). The ethical limits on
human experimentation have made it very difficult to prove
that T reactivity is the sole inciting agent of these
35   diseases. Nonetheless, a large body of experimental work on
animal models -- murine experimental allergic encephalitis as
a model for multiple sclerosis (59, 60), BB diabetic rats for
human diabetes (61, 62), murine collagen-induced arthritis for

rheumatoid arthritis (63, 64), and S antigen disease in rats
and guinea pigs for human autoimmune uveitis (65, 66), among
others -- suggests that T cells are the critical agent of
these diseases. From recent work, the identity of

5   disease-causing proteins or peptide antigens is emerging: i)
multiple sclerosis: the peptide epitopes of myelin basic
protein (MBP) residues 84-102 and 143-168 (45, 66, 67); ii)
autoimmune uveitis: the human S antigen, which has been
recently molecularly cloned (48, 69) ; iii) type II collagen

10   in rheumatoid arthritis (70); and iv) thyroglobulin in
thyroiditis (71). Similarly, a wide variety of proteins have
been identified which stimulate the production of the allergic
immunoglobulin, IgE. IgE is produced by β lymphocytes in a
process that requires lymphokines produced by

15   antigen-activated T cells known as "T cell help."
            The basic concept of the present therapeutic
approach is very simple. Disease-causing T cells are first
challenged by immunization, which causes the activated T cells
to express high affinity IL-2 receptors and to begin producing

20   and secreting IL-2. When the cells are expressing high levels
of IL-2 receptor, additional human IL-2 is infused to very
efficiently drive all the activated cells into cycle. The
cells under the influence of IL-2 are then caused to undergo
apoptosis by re-immunization with antigenic peptide or

25   protein. Further, if the antigen is capable of stimulating
sufficient IL-2 production, it is not necessary to administer
exogenous IL-2. In either case, the timing of rechallenge is
important -- it must occur within a short interval such as 2-3
days after the first stimulus when cells bear the IL-2

30   receptor and are responding to exogenous or endogenous IL-2.
            Protocol:
            As shown in Figure 4, immunization with a specific
peptide or protein is carried out on day one. In the case of
multiple sclerosis, for example, either of two immunodominant

35   peptides from myelin basic protein (MBP) believed to be
encephalitogenic in man, MBP 84-102 (the preferred peptide),
or MBP 143-168 (66-68), that have been coupled to tetanus
toxoid, can be given in alum adjuvant IM, at a dose between

14

about 10 to about 1000 µg. Previous experience using proteins
or peptides has suggested that intramuscular (IM)
administration is optimal (85-87, 89, 90). Newer data suggest
that oral administration may also be effective (73).

5   As with any medicinal substance, or biologic, tests
on any peptides and proteins used for the immunization would
need to be routinely carried out over a range of doses to
determine: 1) the pharmacokinetic behavior of these
substances; 2) their immunogenicity; and 3) safety and
10   identification of any untoward effects. This would constitute
a Phase I clinical trial (84). Thus, the particular proteins
or peptides employed in this protocol (for example, in
multiple sclerosis, MBP 84-102, or MBP 143-168; in uveitis,
the S Antigen; or in rheumatoid arthritis, type II collagen)
15   would require individual routine optimization. Similar
intervention could be used with preparations of
allergy-inducing proteins. These could be derived from a
variety of allergen protein extracts that are now used
clinically, or could be generated by recombinant DNA
20   technology for those such as hornet venom antigen 5, for which
cDNA clones are available (103). Ample evidence from the
development of vaccines suggests that either synthetic
peptides or recombinant DNA-derived proteins are effective in
eliciting an immune response in humans (85-90). These studies
25   also provide guidance as to the range of doses effective for
immunization.

          Proteins:
          1)   Hepatitis B surface antigen, produced as a
recombinant protein in yeast. Adults 2.5 to 20 µg; children
30   1.25 to 5 µg intramuscularly (IM). 90-96% of vaccines showed
an immune response, with the best response at 10-20 µg (85).
Further studies showed the efficacy of a 10 µg dose, with
better results when given IM rather than subcutaneously (86).
20 µg doses in alum adjuvant given IM were found to be
35   effective at preventing infection in clinical trials (87).
          2)   HIV gp 120, either natural or recombinant
molecules. Doses in chimpanzees between 50-1000 µg elicit T
cell responses (88).

Peptides:

1)   Chorionic gonadotropin. Several studies have
indicated successful immune responses against a human
chorionic gonadotropin-β subunit peptide (residues 109-145)
5   coupled to cholera or tetanus toxoid and given in doses from
50-1000 μg in alum adjuvant (89).

2)   Malaria sporozoite antigen. Studies of a
Plasmodium falciparum peptide (NANP)$_3$ coupled to tetanus
toxoid showed an immune response to doses of 20-160 μg of
10   peptide conjugate given IM, with the best response at 160 μg
(90).

Immunization is then followed by a waiting period
during which the antigen activates the subset of T cells
bearing reactive TCRs, causing them to express IL-2 and IL-2
15   receptors. This process will only induce IL-2 receptors on
cells that have been antigenically-stimulated (39). Based on
studies of both human and mouse T cells in vitro, between
about 12 to about 24 hours after antigen exposure are required
to express significant numbers of IL-2 receptors, and as long
20   as about 72 hours are required to express optimal numbers of
IL-2 receptors on the majority of T cells (74; Figure 5).
Thus, the waiting period can be as short as about 12 hours or
as long as about 72 hours, becoming increasingly optimal
toward the upper end of this range.

25   Figure 5: Human peripheral T lymphocytes were
stimulated with either 5 μg/ml concanavalin A or 1 μg/ml
phytohemagglutinin for various time periods. The cells were
then harvested, washed and stained with FITC-labelled
anti-IL-2Rα MAb specific for the human protein (anti-Tac).
30   Flow cytometry was carried out on a Becton-Dickinson FACSCAN
cytometer and analyzed using the Lysis II software.

This is then followed by an infusion of high doses
of IL-2. The administration of high-dose IL-2 to humans has
been well-studied in cancer patients, and various doses have
35   been evaluated (75-79). A number of ongoing protocols
evaluating the medical uses of IL-2 presently exist (95).
Data indicate that IL-2 should be given I.V., either as
frequent bolus doses or as a continuous infusion (75-77).

Doses that have been previously established range between
about 300 to about 3000 units/kg/hour continuous infusion, or
from 10^4 to 10^6 units/kg I.V. bolus (76). Units are defined
by standards available from the Biological Response Modifiers

5   Program at the National Institutes of Health, and are defined
as the quantity of IL-2 that gave 50% maximal thymidine
incorporation in the bioassay under standard conditions. Side
effects of these doses included chills, fever, malaise,
headache, nausea and vomiting, weight gain due to fluid

10   retention, diarrhea, rash, and pruritis, which can all be
treated with acetaminophen or indomethacin; no serious
morbidity or mortality was observed. Despite the apparent
short half-life of IL-2 in serum, at a dose of 3000 u/kg/hr,
IL-2 was detected in patient serum at a level of 5-10

15   units/ml. These levels have been found to predispose on the
order of 60-70% of the T cells to apoptosis, supra. IL-2
infusion can be continued for about 48 to about 72 hours, a
time frame shown to ensure that IL-2 receptor bearing cells
are stimulated into the cell cycle and predisposed to

20   apoptosis (supra, and 74). A 48-72 hour treatment should
avoid the serious complication of excessive fluid retention
even at high doses of IL-2 (76). After IL-2 treatment, the
patient can be immediately reimmunized with an equivalent dose
of antigen. For example, for multiple sclerosis, treatment

25   can be with about 10 to about 1000 μg of peptide 84-102
coupled to tetanus toxoid and given in alum adjuvant IM. It
is likely that the preferred dose would be near the upper end
of this range since greater TCR stimulation produces a greater
level of apoptosis (94). IL-2 treatment would have stimulated

30   the T cells bearing IL-2 receptors -- predominantly the
disease-causing T cells -- and these cells would then be
re-stimulated through their TCR. These cells will then
undergo apoptosis (supra, 74). After an immunization period
of about 12 to about 72 hours, the cycle would begin again

35   with reinfusion of IL-2. As will be described below,
increased efficacy would likely result from multiple cycles of
therapy. The treatment endpoints would be: i) elimination of
in vitro reactivity to the antigen, which can be easily

measured where possible by various mixed lymphocyte or
proliferation assays using peripheral blood lymphocytes; ii)
amelioration of clinical symptoms; or iii) toxicity. The
treatment endpoints for allergic diseases would be: i)

5   improvement of clinical symptoms; ii) normalization of an
allergic skin test; iii) reduction in serum IgE levels; and
iv) where possible to measure, reduced T cell responses to the
allergenic protein.

            Several features of the present therapy require
10   further explanation. First, it is expected that T cells
besides those antigenically stimulated will express high
affinity IL-2 receptors. Treatment with high doses of IL-2
causes expression of the high affinity IL-2 receptor in a
small fraction of resting T lymphocytes (76). However, this

15   should not diminish the specificity of the therapy because
only those cells whose TCRs are stimulated by rechallenge with
antigen will undergo apoptosis, as described supra. The
effectiveness of the therapy could be variable depending on
the nature of the antigen and the exact protocol employed.

20   Extensive in vitro studies indicate that between 50-80% of the
antigen-specific IL-2 stimulated T cells will undergo
apoptosis when rechallenged by TCR stimulation (supra, 76).
Second, the reduction in number of antigen-specific T cells
determines the overall effectiveness of the therapy.

25   Therefore, repeated cycles can substantially increase efficacy
even if the level of killing in each cycle is only 50-70%
(Table 1). As shown in the mouse studies, supra, the level of
antigen-reactive T cells will decrease below the number of
such cells prior to the first immunization with repetitive

30   immunization. Furthermore, the expected toxicity of this
protocol should be minor, and previous studies of the
therapeutic use of IL-2 in humans indicates that all side
effects dissipate promptly following the discontinuation of
IL-2 (75, 76). The most serious side effect, fluid retention,

35   should be minimized by the intermittent nature of IL-2
treatment (79). The 2-3 day rest period between doses would
allow for diuresis of the fluid built up during IL-2
administration. Finally, the repeated administration of

antigen will cause production of some endogenous IL-2, which
will predispose some cells to apoptosis. While it is
extremely unlikely that endogenous levels can reach the very
high levels of IL-2 that can be administered

5   pharmacologically, it is possible that empirically-determined
decreases in the IL-2 dose could be achieved because of
endogenous IL-2 effects. The level of killing is dependent on
the total level of IL-2 to which the T cell is exposed, and
this will reflect a combination of endogenous and exogenous

10   sources (supra, 76).

       With certain antigens, the pre-disposition of cells
to apoptosis may be sufficiently induced by the endogenous
production of IL-2. In these cases, appropriate immunization
with antigen, in the absence of exogenously administered IL-2,

15   could produce T cell apoptosis and a protective effect. Based
on the studies of the timing of susceptibility to apoptosis
disclosed supra, immunizations repeated at specific intervals
would be crucial for effective therapy. To effect
IL-2-mediated apoptosis, immunizations would have to be

20   repeated at about 24 to about 120 hour intervals, preferably
at about 24 to about 72 hour intervals, and would have to be
repeated multiple times. T cell reactivity or cell-mediated
immunity for the specific antigen could then be monitored by
in vitro assays to determine that T cells had undergone

25   apoptosis. Absent the knowledge provided by the discovery
disclosed herein, previous attempts to decrease immune
responsiveness by repetitive immunization have not been
optimal. For example, donor transfusion protocols to
ameliorate graft rejection involved 3 transfusions given at 2

30   week intervals (91, 92). Allergy shots, i.e., desensitization
therapy, are typically given initially at 4-7 day intervals,
after which intervals are progressively increased in length to
2 to 4 weeks (102). Based on the present novel understanding
of T cell apoptosis, the most effective immunization protocol

35   would involve repetitive administrations of antigen at about
24 to 72 hour intervals.

19

TABLE 1

Theoretical number of reactive cells after
fractional killing using IL-2 and T cell
receptor stimulation

Cells Cycle	Fractional Killing	Reactive Remaining
Start	None	100,000
1	70%	30,000
2	70%	9,000
3	70%	2,700
4	70%	810
5	70%	243
6	70%	73

Theoretical values are based on starting with 100,000 cells
and a constant killing efficiency of 70%. A reduction of over
100-fold is seen in 4 cycles and over 1000-fold in 6 cycles.
At a fractional killing of 50%, a reduction of nearly 100-fold
would be seen in 6 cycles.

III. **Method for transplantation antigen/IL-2-mediated apoptosis.**

In medical procedures in which tissue is transferred
between individuals who are genetically non-identical at their
relevant histocompatibility antigen loci, herein referred to
as allografting, and the tissue as an allograft, the major
problem encountered is rejection of the donor allograft by the
host. The term "host" refers to the individual who is the
recipient of the allograft, and the term "donor" refers to the
individual from whom the allograft is derived. Studies of the
process of graft rejection have shown that it is due to the
antigen-specific activation of T lymphocytes, especially those
bearing CD8 surface molecules (80). More importantly, agents
that block the ability of T cells to mount an immune response
in humans effectively prevent or lessen graft rejection (81).

Since CD8^{+} T cells have been shown to be susceptible to apoptosis by IL-2, supra, this phenomenon can be used as a specific means to eliminate the reactive T cells, thereby avoiding graft rejection.

5   Protocol: Essentially the same protocol with respect to timing and IL-2 dose can be used for this therapy as was described supra for the therapy of autoimmune diseases. The major difference between this therapy and that described above is the source of antigen. Major histocompatibility

10   complex (MHC) antigens are cell surface proteins that are tremendously polymorphic among individuals. Each individual's cells bear a genetically determined set, or haplotype, of such antigens which serve as an immunological "fingerprint" on each cell

15   (82). This allows one's immune system, in particular those responses generated by T cells, to recognize one's own cells, and to attack only cells that do not bear the self "fingerprint" (83). There are two classes of MHC -- class I antigens, found on all cells in the body; and class II

20   antigens, found predominantly on monocytes, macrophages, B lymphocytes, dendritic cells, and activated T cells (82). It is the class I MHC antigens that are recognized by CD8^{+} T cells that are the predominant influence in allograft rejection (80, 83). Because of this complexity of MHC

25   antigens, the simplest source is cells from the allograft donor. It has been empirically observed that transfusion of a graft recipient with donor blood suppresses graft rejection, although the mechanism of this effect is unknown, and the clinical effectiveness in many cases is modest (92). These

30   protocols provide evidence that three transfusions of 200 ml of whole blood or packed cell equivalent from the donor is easily tolerated by the recipient with minimal side effects (91). There is evidence that the donor-transfusion in some cases elicited sensitizing antibody responses in the allograft

35   host, and these patients were not given allografts (91). These studies possibly represent an empirical observation that pre-exposure to donor antigen suppresses the T cell response, although this is controversial (93). The present method

includes administration of blood as a source of MHC antigens
in doses of about 50 to about 200 ml to patients in cycle with
IL-2, as indicated in Fig. 4. In the case of kidney
transplants, the amount of blood could be determined by the
5   fluid tolerance of end-stage renal disease patients. The
blood can be given as either whole blood, packed cells, or
washed packed cell transfusions (92). The success of
treatment can be assessed by: i) a decreased requirement for
general immunosuppressive medications; ii) graft survival; and
10   iii) adequate function of the allograft. For example, the
function of a transplanted kidney can be established by
determining serum levels of creatinine and blood urea nitrogen
(104). This can be followed by IL-2 infusion and rechallenge
with blood cells as antigen as shown in Figure 4.

15

IV.   Summary.

The conceptual advance provided by the inventive
discovery that underlies the present methods is that T cell
immunity works as a balance between the production and
20   destruction of antigen-specific T lymphocytes. Previously,
investigators have focused on the use of lymphokine growth
factors such as IL-2 to increase the proliferation and
responsiveness of T lymphocytes (68). It is now proposed that
the opposing T cell mechanisms be used therapeutically. The
25   discovery that IL-2 predisposes T cells to death is contrary
to the previously understood properties of IL-2, and provides
a radically new approach to the treatment of diseases caused
by T cell reactivity. By providing physicians and medical
researchers with the basis of the present inventive discovery,
30   the processes of immune autoregulation leading to T cell
destruction can be exploited in combatting disease.

It has been previously known for some time that
prior activation and IL-2 production were capable of
diminishing immune responsiveness both _in vivo_ and _in vitro_
35   (1-4, 95-97). The mechanism for these effects was not
understood. Absent the knowledge that IL-2 predisposes T
lymphocytes to antigen-dependent apoptosis, it was not
possible to manipulate this phenomenon for medical or

therapeutic purposes. Recent results demonstrate that human T
lymphocytes are quite susceptible to apoptosis following IL-2
exposure (74). It is now possible to rigorously study the
kinetics and dose requirements of IL-2 in the predisposition

5   phase, and antigen in the apoptosis phase, to routinely
optimize the treatment cycle for a given disease following the
guidance provided herein.

    That this process depends on the discovery of a
novel property of IL-2 is particularly auspicious. IL-2 is

10 perhaps the best studied lymphokine (68). It is
well-understood genetically, its cDNA and gene have been
molecularly cloned, and its mRNA expression has been
thoroughly studied (68). IL-2 is already available
pharmaceutically in a form for use in humans (79). Previous

15 studies in human cancer victims, detailed above, have given
clear insights into how IL-2 affects human physiology at
different doses (79). All of these features significantly
enhance the feasibility of its novel use to cause
auto-destruction of disease-causing T lymphocytes for the

20 treatment of a wide variety of diseases in humans and other
mammals.

                            Example 1
        This example shows the effect of IL-2 on antigen
response in A.E7, a non-transformed CD4$^+$ T_H1 T lymphocyte

25 clone that constitutively expresses high affinity IL-2
receptor and produces IL-2 after antigen stimulation (10,11).
Resting A.E7 cells given 1 μM antigen underwent proliferation
due to endogenous IL-2 production (from 435 to 22894 CPM
[^{3}H]thymidine). By contrast, A.E7 cells given 100 units/ml

30 exogenous IL-2 for two days and then 1 μM antigen showed
decreased [^{3}H]thymidine incorporation (51755 to 7140 CPM).
Decreased incorporation might have been due to an
antigen-dependent block in IL-2 stimulated proliferation
(12,13), but microscopic examination unexpectedly revealed

35 extensive death of the T cells (Fig. 1A, upper panels). For
the IL-2 pretreated sample, quantitation revealed 82% fewer T
cells following 1 μM antigen stimulation compared to control
Table 2). Cell death was less dramatic with lower doses of

IL-2, but was still evident between 2 and 5 nits/ml, at
which 50-60% of the T cells were killed (Table 2). A smaller
cell loss was seen in T cells given no IL-2 pre-treatment that
could be attributed to IL-2 produced by antigen stimulation.
These results suggested the hypothesis that IL-2 following
antigen stimulation leads to proliferationt, whereas IL-2
exposure prior to antigen stimulation causes cell death.

Table 2 Effect of IL-2 and antigen receptor stiumlation on T cell viability

Cells	Pretreatment	Cell Number/well (x10^4) Control	1μM antigen	stimulated % control
A.E7 Expt 1	no IL2	3.8±0.3	2.3±1.8	60%
	100 units IL2	4.9±2.5	0.9±0.6	18%
Expt 2	no IL2	3.6±0.4	2.8±0.6	78%
	2 units IL2	5.0±0.4	2.4±0.1	48%
	5 units IL2	6.2±0.5	2.4±0.4	39%
	10 units IL2	6.8±0.6	2.0±0.2	29%
	50 units IL2	6.8±1.0	2.0±0.7	29%
		Control	10 μg/m anti-CD3ϵ	
Expt 3	no IL2	4.3±0.5	3.2±0.4	74%
	2 units IL2	7.1±1.5	3.6±0.1	51%
	5 units IL2	6.4±0.8	2.1±0.6	33%
	10 units IL2	7.4±1.5	1.0±0.2	14%
		Anti-CD3ϵ	Anti-CD3ϵ+CsA	
Ept 4	no IL2	3.9±0.5	4.4±0.5	
	25 units IL2	2.2±0.5	4.2±0.9	
		Control	20 μg/ml anti-Vβ8	
LNT	3 units IL2	5.7±1.1	9.1±1.2	160%
	100 units IL2	39.8±2.0	20.1±6.4	50%
			33 μg/ml anti-Vβ6	
	3 units IL2		9.4±1.0	165%
	100 units IL2		26.8±4.7	67%

5   Cell counts (x10⁻⁴) are averages of 4-6 independent
hemocytometer counts of three wells determining only
trypan-blue excluding cells. Antigen was the 81-104 peptide
from pigeon cytochrome c (a gift of B. Beverly). The
anti-CD3ε antibody 145-2Cl1 (16) was used at the
10   concentrations indicated, except for Expt. 4 where 2.5 µg/ml
was used. Plates were coated with 20 µg/ml anti-Vβ8 (F23.1
MAb) and 33 µg/ml anti-Vβ6 MAb(RR4-7MAb) as described in the
legend to Figure 1. Control experiments in which equivalent
amounts of MAb recognizing CD4, MHC class I, or CD45 were
15   coated on plates had no effect on cell viability (data not
shown). Cells were incubated in dishes for 48 hours. Trypan
blue stained cells (blue) made up 30-70% of the differences
between stimulated and controls where quantitated.
Cyclosporin A (CsA, a gift from Sandoz Pharmaceuticals, Inc.)
20   was included at 100 ng/ml only during the stimulation by
145-2Cl1 antibody. IL-2 was human recombinant IL-2 (provided
by Dr. Craig Reynolds, Biological Response Modifiers Program,
NCI) or supernatant from MLA-144 cells (provided by the
Fermentation Laboratory, FCRF, NCI), both of which gave
25   essentially identical results. Data are representative of 8
experiments.

       To test this idea, IL-2 and antigen stimulation were
30   evaluated in an experiment in which endogenous IL-2 was not
produced. A.E7 cells (and other CD4⁺ T_H1 T cell clones)
require a co-stimulatory signal from antigen-presenting cells
(APCs) in addition to occupancy of the T cell receptor complex
to produce IL-2 (14,15). Therefore, in the absence of APCs,
35   A.E7 cells were pre-treated with IL-2, washed, and stimulated
on culture dishes coated with a monoclonal antibody (MAb) to
CD3ε complex (16). This resulted in almost no endogenous IL-2
production (data not shown). Nonetheless, IL-2 pre-treatment
followed by anti-CD3ε stimulation again led to extensive T
40   cell death (Fig. 1A, lower panels). Quantitation showed that
74% of the untreated cells, but only 14% of the T cells pre-
treated with 10 units/ml of IL-2, were recovered alive (Table
2). As was observed with antigen stimulation, killing caused
by anti-CD3ε was dependent on the IL-2 dose, with 49% cell
45   loss at 2 units/ml. Dying A.E7 cells exhibited a pattern of
DNA fragmentation to 200 bp nucleosome-length multiples after
IL-2 and anti CD3ε stimulation (Fig. 1B, 2Cl1). Also, cell

death was abrogated by cyclosporin A (Table 2, CsA).
Together, these data strongly suggested that apoptosis was
occurring (1,2,5,7).

It was then tested whether IL-2 could predispose
cells bearing particular T-cell receptors (and not bystander
cells) to apoptosis in a heterogeneous lymph node T (LNT) cell
population. Because LNT cells do not constitutively express
IL-2 receptors, they were first stimulated with the lectin
concanavalin A. This caused the cells to express the IL-2
receptors and become IL-2-responsive (data not shown). The
concanavalin A was then removed and the cells were exposed to
IL-2. Since LNT cells did not survive without any IL-2, low
dose (3 units/ml) and high dose (100 units/ml) IL-2 were
compared. IL-2 was given for two days and the LNT cells (>97%
$\alpha\beta$ T cells, data not shown) were plated on dishes coated with
either no antibody, the F23.1 monoclonal antibody (MAb) (anti-
Vβ8, specific for the Vβ8.1,2,3 receptor chains) (17), or the
RR4-7 MAb (anti-Vβ6) (18). In low IL-2, both anti-Vβ8 and
anti-Vβ6 MAbs caused the cell number to increase (Table 2).
After high IL-2, anti-Vβ8 caused nearly 50% cell loss, and
anti-Vβ6 led to a 33% cell loss (Table 2) . Flow cytometry
revealed that anti-Vβ8 MAb markedly deleted cells with cognate
Vβ8 receptors in LNT cells given high IL-2 but not in LNT
cells given low IL-2 (data not shown). To accurately
quantitate the deletion observed with high dose IL-2, the
populations were gated separately into CD4$^+$ cells and CD4$^-$
cells (virtually all CD8$^+$ cells, see legend)(Table 3).
Anti-Vβ8 MAb decreased the fraction of Vβ8$^+$ cells from 38.4%
to 14.1% for CD4$^+$ cells and from 38.0% to 19.4% for CD4$^-$(CD8)
cells but had no effect on Vβ6$^+$ cells, which were relatively
increased to compensate for the loss of Vβ8$^+$ cells (Table 3).
Similarly, anti-Vβ6 MAb caused deletion of Vβ6$^+$ cells (from
12.3% to 1% for CD4$^+$ cells and from 10.0% to 2.2% for CD4$^-$
(CD8) cells), but not Vβ8$^+$ cells, which were relatively
increased (Table 3). These findings were not due to T cell
receptor down-modulation because: 1) substantial apoptosis
and decreased cell number were observed; 2) cells bearing
heterologous receptors were relatively increased; and 3) no T

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cell receptor negative cells were detected (data not shown).
Thus, IL-2 predisposes to an endogenous death pathway in both
$CD4^+$ and $CD8^+$ T cells. Bystander cells, though competent to
undergo apoptosis, are not affected by
5   antigen-receptor-mediated killing of a subpopulation of LNT
cells.

TABLE 3   Flow cytometric quantitation of in vitro deletion of Vβ8- and Vβ6-
bearing LNT cells using anti-receptor antibodies

Gating	Fraction of total gated cells positive for:	Stimulation after LI-2 pre-treatment		
		None	Anti-Vβ8	Anti-Vβ6
CD4+cells	Vβ8	38.4%	14.1%	3%
	Vβ6	12.3%	15.9%	1.0%
CD4-(CD8) cells	Vβ8	38.0%	19.4%	40.6%
	Vβ6	10.0%	16.4%	2.5%

Lymph node T cells pre-treated with 100 units/Ml IL2 were prepared as described in Fig. 2 and stimulated on culture dishes coated with either no antibody or MAb against either Vβ8 (F23.1) or Vβ6(RR47). Cells recovered from the plates were stained for two color cytometry with both anti-CD4 (Becton Dickinson) and either anti-Vβ8(F23.1) or anti-Vβ6 (RR4-7). The CD4 staining was used to gate the cells into CD4^{+} and CD4^{−} pools; control staining showed that virtually all CD4^{−} cells were CD8^{+} (using anti-Lyt2), and cells were >97% αβ T cells (using H57-597) in these preparations. The gated pools were then quantitated by flow cytometry using a Becton Dickinson FACSCAN for the fraction of either Vβ8[+] or Vβ6^{+} cells. Independent gating was necessary for accurate quantitation because of the previously described overgrowth of CD8 cells in antibody stimulated samples (29). The fraction of the gated pool that was positive for either Vβ8 or Vβ6 is given as percent; boxed values show Conditions where deletion was observed. The data are representative of 5 experiments.

The hypothesis that IL-2 preceding antigen receptor occupancy leads to apoptosis predicted that repetitive immunization could eliminate antigenspecific T cell clones _in vivo_. Furthermore, such elimination would depend on IL-2

5   produced by activated T cells predisposing themselves and their progeny to death. To test this prediction, BALB/c mice were given Staphylococcus aureus enterotoxin B (SEB) I.V. using a loading dose of 500 μg followed by two injections of 125 μg at two day intervals. SEB was used because it

10   activates all T cells bearing a Vβ8 polypeptide chain in their T cell receptor. Vβ8 bearing cells comprise nearly one quarter of the repertoire of a BALB/C mouse, and therefore can be measured easily by flow cytometry. After eight days, the mice were sacrificed, and peripheral lymph node T cells were

15   analyzed for Vβ8$^+$ cells (which are specifically activated by SEB) and Vβ6$^+$ cells (which are not stimulated by SEB) (19). Flow cytometry for representative mice is shown in Fig. 2. In an uninjected animal, 22.3% of the T cells were detected by the antibody KJ16-33 (20) which recognizes Vβ8.1,8.2

20   receptors. As predicted, repetitive immunization with SEB reduced the relative number of Vβ8.1,8.2$^+$ cells to 7.5%, over a 60% decrease. Injection of SEB together with 800 μg of MAb 3C7, an IL-2 receptor alpha chain blocking antibody (21,22), (anti-IL-2R, a gift of Dr. A. Kruisbeek) every 12 hours I.P.

25   caused a striking reversal of the loss of Vβ8.1,8.2$^+$ cells to 18.1%. Co-injection of MAb 11B11 previously used to block IL-4 responses _in vivo_ (22,23) (anti-IL-4, a gift of Dr. W. Paul), did not reverse the loss of Vβ8.1,8.2$^+$ cells caused by SEB (Table 3). The fractions (mean ± S.D.) of Vβ8.1,8.2$^+$

30   cells for several mice were similar: normal, 23.3 ± 0.6% (n=4), SEB only, 9.7 ± 2.8% (n=4), SEB + anti-IL-2R, 19.5 ± 3.8% (n=3), and SEB + anti-IL-4, 9.4 ± 1.8% (n=3). Vβ6$^+$ T cells showed no deletion in these mice. A similar blocking effect was observed using the MAb S4B6 that directly binds the

35   IL-2 lymphokine molecule itself (data not shown). No effects on the number of Vβ8 or Vβ6 cells were seen if antibody 3C7 or 11B11 was injected without SEB (data now shown). Moreover, no evidence of Vβ8 T cell redistribution from lymph into other

tissues was found by pathological analysis (data not shown).
Thus, clonal elimination caused by SEB under these conditions
depends on IL-2 but not IL4.

5   By three different experimental protocols, a direct
involvement of IL-2 in antigen-receptor driven T lymphocyte
elimination was found. IL-2-induced apoptosis has the
features of feedback inhibition (25) it is caused by an
"end-product", e.g., IL-2, of the initial antigen stimulation;
ii) apoptosis was greater with increasing doses of IL-2; and
10   iii) it reverses the increased T cell numbers initially caused
by antigen (1,4,8,9). T cell clonal specificity is maintained
by the requirement for antigen stimulation as well as IL-2 for
apoptosis; however, antigen receptor occupancy alone is not
sufficient for apoptosis. A useful term for this feedback
15   pathway would be "propriocidal" regulation (Latin: proprius,
"one's own") to indicate selective killing of the stimulated T
cells, their progeny, and clones of related specificity. One
conceivable role of this pathway may be illustrated by
Staphylococcal enterotoxins whose lethality seems due to
20   substances produced by activated T cells (19,26).
IL-2-mediated apoptosis could eliminate the affected T cells
and decrease the harmful effects of chronic exposure to these
toxins.

These results have been extended to human T
25   lymphocytes (74). Human peripheral blood T lymphocytes were
stimulated to express the high affinity IL-2 receptor using
either phytohemagglutinin or concanavalin A. These cells were
then stimulated with either 0, 2, or 200 units of IL-2.
Proliferation and an increased cell number were observed in
30   response to IL-2 (Fig. 3). Upon rechallenge of the cells with
an antigen surrogate, namely, a MAb against a human CD3
polypeptide of the T cell receptor (OKT3), the cell numbers
dropped if the cells had previously been exposed to IL-2 but
not if they were untreated (Fig. 3). In samples pretreated
35   with IL-2 and then restimulated with OKT3, ladders of
fragmented DNA were also observed by agarose gel
electrophoresis, indicating that apoptosis was occurring (data
not shown). Thus, the ability of IL-2 to predispose to

apoptosis is not a peculiarity of murine T cells, but also
extends to human T lymphocytes, and most likely represents an
intrinsic T cell regulatory mechanism. This therefore makes
it possible to exploit antigen driven T cell apoptosis for the
5   treatment of diseases in humans, mice, and presumably mammals
or other animals that have T cells with similar properties.

Figure 1: The A.E7 T cell is a non-transformed CD4$^+$
T_H1 T cell clone that produces IL-2 after stimulation by a
pigeon cytochrome C peptide (amino acids 81-104) in the
10   context of E^k that was carried as described previously
(11,14). Lympholyte-M purified A.E7 T cells were pre-treated
for 48 hours with MLA144 gibbon ape leukemia cell supernatant
to provide 100 units/ml of IL-2 activity. T cells were
harvested, washed 3 times with medium (Click's medium with 10%
15   fetal calf serum, 2 mM glutamine, and 50 μM β-mercaptoethanol
added; Biofluids, Inc.) and stimulated in 96-well dishes.
Antigen stimulations contained 2 X 10^4 T cells, 1 X 10^4 DCEK
(E_α, B_β^K - expressing L cell transfectants, a gift of Dr.
Ronald Germain, NIH) APCs given 3000 Rads, and I μM purified
20   pigeon cytochrome C peptide (amino acids 81-104) in 200 μL
total volume. For thymidine uptake, after 24 hours, 0.5 μCi
of [^{3}H]TdR (Amersham) was added, incubation was continued for
8 hours, and samples were then assayed by scintillation
counting. For photomicroscopy, after 40 hours of stimulation,
25   the medium was replaced with 0.4% trypan blue in phosphate
buffered saline (PBS, 0.8 mM potassium phosphate, 154 mM
sodium chloride, and 2.9 mM sodium phosphate, pH 7.4) for 10
minutes. The stain was removed and the wells gently washed
three times with PBS only. Photomicrographs were made on a
30   Zeiss Axiovert 405 M microscope using Hoffman modulation
contrast optics. Antibody stimulations used 96-well plastic
culture dishes coated with 10 μg/ml solutions of protein A
column-purified anti-CD∈MAb (145-2C11) (15) in PBS for 4 hours
at 370C. Wells were washed two times with PBS, once with
35   medium, and filled with 5 X 104 A.E7 T cells in 200 μL of
medium. Lymph node T cells (for Tables 2 and 3) from
axillaryl inguinal, and mesenteric nodes excised from BALB/c
mice were placed into medium containing 3 μg/ml concanavalin A

for 48 hours, then treated with 10 µg/ml α-methylmannoside
for 30 minutes, washed extensively, and placed in culture for
48 hours with either 3 or 100 units of Il-2. Antibody
stimulations (F23.1 and RR4-7) were in 75 cm^2 culture flasks

5   coated with antibodies as in Fig. 2 and inoculated with 1 x
10^7 cells in 12 mls medium containing either 5 or 10^7 cells in
12 mls medium containing either 5 or 100 units IL-2/ml. Cells
were harvested, isolated by Lympholyte-M, and stained for
cytometry. DNA preparations of A.E7 cells stimulated with IL-

10 2 and anti-CDϵ (scaled to 5 mls) were carried out as described
previously (5).

        Figure 2: Vβ8 samples (left panels) were stained
with MAb KJ16-133 (20) which detects Vβ8.1,8.2 TCRs and Vβ6
samples (right panels) were stained with MAb RR4-7[18] for Vβ6

15 TCRs. Histograms are relative fluorescent intensity versus
cell number; positive cells, as gated by the dotted line, are
percentages of total T cells. Female, six-week-old BALB/c
mice were injected with Staphyloccocal entertoxin B (Sigma)
diluted in 250 µL sterile 1X PBS in the tail vein as follows:

20 day 0 - 500 µg, day 2 - 125 µg, and day 4 - 125 µg. Lymph
node T cells did not express IL-2 receptor α chain until 12
hours after the first SEB injection; therefore, I.P.
injections of 800 µg of MAb 3C7 were initiated 12 hours after
the first SEB dose, and given every 12 hours until the

25 experiment ended. MAb 3C7 has been shown to block IL-2
responses in vitro (ref. 21, 22 and M.J.L., unpublished
results). MAb 11b11 was given similarly in 1 mg doses. Each
MAb injection was given in 300 µL of 5% dextrose in water,
which provided a simple metabolite and hydration to prevent

30 mortality of the mice during the course of each experiment.
After eight days, the mice were sacrificed, and lymph node
cells were directly stained. Staining for flow cytometry was
carried out in 1X PBS with 0.1% bovine serum albumin and 0.1%
sodium azide with pre-determined dilutions of primary antibody

35 (mouse immunoglobulin (Ig) γ2a MAb F23.1 or rat IgG2b,
KJ16-133 for Vβ8 or rat IgG2b RR4-7 for Vβ6) followed by
either a fluorescein isothiocyanateconjugated goat anti-mouse
IgG2a (Southern Biotechnology) or a goat F(ab')$_2$ anti-rat IgG

32

H and L (Caltag Laboratories). CD4 was detected with
phycoerythrin-conjugated anti-L3T4 antibody (Becton -
Dickinson). Minor residual dead cells were gated by propidium
iodide. Samples were 50,000 events analyzed with 3 decade
5   logarithmic amplification on a Becton Dickinson FACS 440 dual
laser cytometer interfaced to a Digital Equipment Corporation
PDP 11/24 computer and plotted as isocontours of total cell
number.

Figure 3: Human peripheral blood mononuclear cells
10   were purified by Ficoll density gradient centrifugation from
blood packs obtained from anonymous donors through the
Department of Transfusion Medicine at the National Institutes
of Health. The cells were incubated in RPMI 1640 medium with
10% fetal calf serum, and aliquots of cells were given 5 μg/ml
15   concanavalin A for 2-3 days. Cells were then harvested,
quantitated, and incubated in flat-bottom plastic dishes
precoated with either 0, 1, 5 or 50 μg/ml OKT3 MAb. After 2-3
days, 4-6 cell counts were performed and averaged for each
point.

20   The findings described above establish a direct role
for IL-2 in clonal elimination of mature T cells, which is
postulated to be a mechanism of extra-thymic tolerance
(1,4,8,9). Recently, Kawabe and Ochi have shown that the loss
of mature Vβ8 cells following SEB injection is the result of
25   apoptosis (1). This study demonstrates that loss of Vβ8$^+$ T
cells may be faster and greater in magnitude if larger amounts
of SEB are repeatedly administered. This would support the
model that antigen re-stimulation of T cells under the
influence of IL-2 will cause apoptosis.

30   How does IL-2, which is well known for its mitogenic
effect on T lymphocytes, paradoxically program the same
cell-type for apoptosis? One possibility is that IL-2 serves
only to drive T cells into the division cycle, which has been
recently suggested to pre-dispose thymocytes and $\gamma\delta$ T cells to
35   death (27,28). If this is true, then any successful immune
response could pre-dispose mature $\alpha\beta$ T cells to apoptosis.
Alternatively, IL-2 could provide a qualitatively or
quantitatively distinct signal that entrains apoptosis to

antigen receptor stimulation. in either case, in evaluating
IL-2 as a therapeutic agent in humans, it will be important to
consider its unexpected ability to pre-dispose T cells to
apoptosis.

5   EXAMPLE 2

This example shows that T cell death can be caused
by stimulation with high doses of antigen without exogenously
administered IL-2.   In particular, the data presented here
show that the T cell death depends on IL-2 produced by the T

10   cells.

Figure 6 shows results of experiments in which the
non-transformed, CD4+ T_H1 T lymphocyte clone A-E7 was
stimulated with increasing concentrations of its cognate
peptide -- pigeon cytochrome c amino acids 81-104. $1x10^4$

15   A-E7 lymphocytes were stimulated with peptide antigen using
$5x10^5$ irradiated syngeneic splenocytes as antigen presenting
cells in flat bottom 96 well plate.   Samples were incubated
for 72 hours then pulsed with 1 μCurie of ^{3}H-thymidine for
18-20 hours.

20   Figure 6 shows that proliferation, as measured by
incorporation of [^{3}H]-thymidine (line with squares), peaks at
0.01 μM and then decreases at higher doses.   At the greatest
antigen dose of 10 μM proliferation is 10% of the maximum.
Despite the apparent proliferative blockade, production of

25   interleukin-2 (IL-2) is maximal in the suppressive range (line
with circles).

These results demonstrate that supraoptimal
concentrations of antigen cause suppression of ^{3}H-thymidine
incorporation in the presence of maximal IL-2 production.

30   The results presented in Figure 7 show that T
lymphocytes die at high antigen doses.   A-E7 lymphocytes
($5x10^4$ per well) were incubated with no (left panel), 0.1 μM
(middle panel) or 10 μM (right panel) pigeon cytochrome c
peptide in the presence of 50 fold excess of irradiated DCEK

35   antigen-presenting cells.   Following a 48 hour incubation the
cultures were stained with trypan blue for 10 minutes, rinsed,
then photographed using Hoffman modified optics in a Zeiss
Axiovert 405 M microscope.

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Cells appearing dark are unable to exclude the dye
and thus are non-viable. As can be seen, the 10 μM sample had
a significant number of dead cells whereas at low or 0 antigen
doses very few cells are dead. These results demonstrate that
5   high antigen doses cause significant T cell death.

The results in Figure 8 show that decreased T cell
number quantitatively accounts for the suppression of ^{3}HTdR
cpm.

In order to quantitate the amount of death occurring at high
10   antigen dose a FACS viability assay was devised. T
lymphocytes (1×10^4 A-E7) were incubated at the indicated
concentration of their cognate antigen with 50 fold excess of
syngeneic splenocytes for 72 hours. The samples were
harvested, rinsed, then stained with a fluorescein labelled
15   anti Vα11 antibody and anti-CD4 antibody in order to determine
the fate of the A-E7 cells following stimulation. In
addition, samples were stained with propidium iodide as a
means of excluding non-viable (propidium iodide positive)
cells. Panel a. is a representative experiment in which CD4+
20   cells are analyzed for the presence of Vα11 T cell receptor.
The experiment shows an antigen specific increase in cell
number at 0.01 μM. At higher doses, however, cell number is
dramatically reduced. The 10 μM sample has 80% fewer viable
cells than the 0.01 μM point. Moreover, at 10 μM there are
25   20% fewer cells than the 0 antigen point, demonstrating a
deletion below the baseline number of cells added to the
experiment.

In order to determine if antigen specific cell death
would occur in primary T lymphocytes, the same experiments
30   were conducted in lymph node cells harvested from mice
carrying the transgenic construct for the myelin basic protein
T cell receptor (Vα2.3;Vβ8.2) which recognizes the myelin
basic protein peptide Ac1-11. Cells were stimulated with the
indicated concentrations of Ac1-11 for 5 days then stained for
35   analysis with anti Vα2.3 antibodies and anti-CD4 antibodies.
Panel b. is a representative experiment showing the antigen
specific loss of viable T lymphocytes at high antigen
concentrations. In this case, the 100 μM sample has 95% fewer

cells than the 1.0 μM point. These experiments demonstrate
that antigen specific death of both T cell clones and primary
lymphocytes can account for the observed high dose suppression
of proliferation.

5   The results in Figure 9 reveal that endogenous
production of IL-2 is necessary and sufficient for antigen
specific T cell death. Using the FACS viability assay
described above, cell number for A-E7 lymphocytes was
quantitated at increasing concentrations of antigen either
10   with or without inclusion of 3C7, an antibody which binds the
alpha chain of the IL-2 receptor and blocks IL-2 bioactivity.
At 10 μM peptide antigen there were 75% fewer live cells as
compared to the maximal concentration. In the presence 3C7
this reduction in cell number was abrogated. These results
15   demonstrate that specifically reactive T cells can be deleted
at high antigen doses without the requirement of exogenous IL-
2, and that this deletion is blocked when IL-2/IL-2R
interaction is disrupted.

            The data presented in figures 6-9 demonstrate that
20   high dose suppression can be caused by propriocidal death, a
mechanism which is initiated under conditions of strong TCR
stimulation (such as high concentrations of antigen) and IL-2
from either endogenous production of from exogenous sources.

25   EXAMPLE 3
            This example provides evidence that repetitive
administration of antigen to animals causes deletion of
specifically reactive T lymphocytes in vivo without affecting
non-reactive "bystander" cells and that deletion of the
30   pathogenic clones ameliorates disease.
            In particular, this example provides evidence
demonstrating the efficacy of the present invention in animal
models for multiple scelorosis, autoimmune uveitis disorders,
and allergic response
35

Experimental Allergic Encephalomyelitis
            Experimental allergic encephalomyelitis (EAE) is
considered by those skilled in the art of neuroimmunologic

diseases to be an animal model of neuroimmunologic diseases
such as multiple sclerosis and acute disseminated
encephalomyelitis. Agents that suppress animal EAE lesions
are considered to be of potential clinical utility in the

5   treatment of neuroimmunologic diseases. For example, a number
of cytotoxic agents have been shown to suppress EAE lesions in
animals. Some of these agents have shown clinical efficacy in
patients with multiple sclerosis.

Figure 10 describes the protocol for induction and

10   treatment of EAE. Donor mice were immunized with 400 µg
myelin basic protein (MBP) in complete Freund's adjuvant Ten
days later, draining lymph nodes were harvested, then made
into a cell suspension and stimulated with myelin basic
protein in vitro to further increase the frequency of MBP

15   responsive cells. The cells are rinsed then injected into
recipient mice. Symptoms of disease ensued 6-9 days later
often beginning as tail paralysis that progressed quickly to
hind limb then complete body paralysis. The disease follows a
remitting and relapsing course.

20   The clinical grading of the disease in the following
experiments was as follows: 0 - normal; 1 - limp tail; 2 -
moderate hindleg paresis; 3 - sever hindleg paresis; 4;
hindleg paralysis; 5 - whole body paralysis; 6 - death.

Figure 11 shows the results from experiments using

25   repetitive injections of MBP and IL-2 to prevent EAE. 400 µg
MBP was administered intravenously twice daily on days 0, 2
and 4 post transfer. 30,000 units of IL-2 was administered
twice a day on days 0-4. The mice (5 mice/group) were
followed for 65 days. The data demonstrate that the MBP/IL-2

30   therapy dramatically reduces the severity of the disease as
seen by the reduction in mean clinical score. In addition,
the incidence of disease was lowered since 100% of untreated
mice got sick whereas only 40% of treated mice developed the
disease.

35   Figure 12 shows that repetitive injections of 400µg
MBP can prevent progression of early stage disease. In this
experiment MBP administration was initiated on day 9 post
transfer when the first mouse developed symptoms of disease.

At this point all the mice in the group were treated with MBP
then again on days 11, and 13. The data show a significant
difference on severity of disease. Also, only 20% of treated
mice developed disease whereas 100% of untreated animals got

5   sick. The experiment demonstrates that the antigen therapy
can abrogate progression of early stages of disease.

    Figure 13 shows that repetitive injections of 400μg
MBP blocks relapses of disease, thus demonstrating the
effectiveness of this therapy to ameliorate existing disease.

10   Following the first remission the mice were split into 2
groups: treatment and no treatment. The treatment group
received 2 i.v. injections of MBP on days 17, 19, and 21 post
transfer. The graph shows that the untreated animals,
following their remission progressed to have sustained

15   relapses. The treated mice experienced no such relapse,
demonstrating that the therapy abrogates progression of
chronic disease.

    Figures 14 and 15 present the results of experiments
which correlate amelioration of disease with activation of the

20   propriocidal mechanism by showing antigen specific deletion of
pathogenic T lymphocytes in vivo.

    The first experiments involved repetitive doses of
MBP antigen delete in vivo mature T lymphocytes stained with
the vital dye DiI. As discussed above, following priming of

25   donor mice, lymphocytes are further activated in vitro with
MBP. Prior to transfer the cells were stained with the vital
dye DiI, which is stably incorporated into the plasma
membrane. The dye, when excited at the proper frequency has a
characteristic emission pattern that can be detected by FACS

30   thereby allowing detection of cells stained with the dye. The
dye is known to be stable for greater than 4 weeks which
allows ample time to transfer stained cells, treat mice, then
harvest lymph nodes and spleen to analyze for the presence of
encephalitogenic lymphocytes which would be DiI positive.

35   Figure 14 shows that animals which received stained
cells without therapy get disease as predicted (grade 3),
whereas animals treated with MBP did not get sick. As a
control for antigen specificity, 400μg ovalbumin was

administered to another group of mice. Ovalbumin treatment
provided no protection from disease. To determine if antigen
specific deletion occurred we analyzed by FACS the spleens of
each mouse collecting all CD4+ lymphocytes then analyzing for
5   the presence of DiI. As indicated in Figure 14 non-treated
animals had the highest frequency of CD4+/DiI+ cells. In
contrast MBP treated mice had levels of DiI signal comparable
to untreated mice suggesting that the reduction the in number
if DiI+ cells was MBP specific.
10   Figure 15 presents the results of experiments
showing that repetitive doses of MBP antigen delete MBP
transgenic lymphocytes in vivo. In these experiments, TCR
transgenic lymphocytes were activated in vitro by their
cognate peptide Ac1-11 and transferred into syngeneic mice.
15   As show in Figure 8, these lymphocytes undergo death at high
concentrations of antigen. The transgenic TCR were detected
using antibodies specific for the receptor.
                The mice received 3×10^7 activated lymphocytes and
then treatment with 400μg of either MBP or ovalbumin twice a
20   day i.v. on days 0, 2, and 4 post transfer. A third group
received no treatment. Figure 15 demonstrates, first al all,
that the transgenic cells can induce disease; untreated mice
developed grade 4 disease.
                In order to correlate level of disease with
25   frequency of reactive cells lymphocyte preparations from
mesenteric lymph nodes were made. Three color staining was
preformed with antibodies against CD4, Vα2.3 and Vβ8.2. For
FACS analysis only CD4+ cells were collected. The FACS plots
in Figure 15 show CD4+/Vβ8.2+ cells then plotted for the
30   frequency of Vα2.3 positive cells. The highest frequency of
cells was seen in the untreated animals, which correlates well
with the level of disease which was the highest of the tested
groups. In contrast, mice treated with MBP had 62% fewer
Vα2.3 than the untreated groups demonstrating a significant
35   deletion of reactive T cells. Mice treated with ovalbumin had
no such deletion nor were they protected from disease
demonstrating that the effect is antigen specific.

Experimental Allergic Uveitis

The present invention can be used for targeted T
lymphocyte deletion with other antigens, as shown using a
different autoimmune model-- experimental allergic uveitis
5   (EAU) a mouse model for human autoimmune uveitis disorder.

The protein which causes EAU has been isolated, it
is interphotorecptor retinol-binding protein (IRBP) a protein
found in the retina. EAU is initiated by inoculation of
genetically susceptible mice with an emulsion of 50 μg IRBP
10   and complete Freund's adjuvant (CFA). 12-18 days post
inoculation ocular inflammatory infiltrates ensue. After 3-4
weeks scarring with resultant permanent damage occurs.
Definitive scoring of disease requires histopathology.

To demonstrate the clinical efficacy of propriocidal
15   regulation in therapy for EAU a protocol as described in
Figure 16 was devised. Mice were initially primed with an
IRBP/CFA emulsion on day 0, then split into different
treatment groups. Groups received a total of 4 repetitive
injections of IRBP in incomplete Freund's (IFA) either qD
20   (each day) for first 4 days, or q3D (every third day).
Additionally, certain groups received concomitant injections
of 30,000 units IL-2 or IL-2 alone that began day 3 post
inoculation and ran through day 12.

Figure 17 shows scoring of EAU by ocular
25   histopathology. Scoring was performed as described in Caspi
et al., J. Immunol. 136:9928-9933 (1986). Mice 1-4 which
received only vehicle with no IRBP, showed no signs of
disease. IRBP alone without further injections (mice 5-7) had
the most significant disease ranging in severity from grade 1-
30   3. In the treatment groups, IL-2 seemed to have no protective
effect when administered without IRBP. In all groups
receiving repetitive injections of IRBP, mice were somewhat
protected from disease as compared to the untreated controls.
Interestingly, the timing of the repeat doses had a
35   significant effect since groups treated q3D have less severe
disease than untreated controls as well as the qD treatment
group. Addition of exogenous IL-2 to this regimen resulted in
the most dramatic protection. Mice 23-25 which received IRBP

q3D and IL-2 are disease free, indistinguishable from unmanipulated normal animals.

Allergic Responses associated with IgE

5   Figure 18 shows the schedule of immunization and treatment in the production of serum IgE in response to chicken egg albumin. To immunize for the production of serum IgE, 10 μg of chicken egg albumin was given in alum adjuvant IP in BABB/C mice. To treat the IgE response to this immunization, 0.5 mg of chicken egg albumin in phosphate-
10   buffered saline was given IP on days 1, 3, and 5. In a separate treatment trial, animals were given the same immunization and treatment with chicken egg albumin, but were also given 2 doses of 1x10^4 units of human recombinant IL-2 each day from days 1 through 5.

15   Figure 19 shows the measurement of serum IgE. The measurement of serum IgE in each group of four mice revealed significant production of IgE in the animals that received the immunization with chicken egg albumin but no treatment (Group 1, n=4). Treatment with either antigen alone (Group 2, n=4)
20   or with antigen + IL-2 (Group 3, n=4) both led to dramatic reductions in the level of IgE with a slightly better response for the latter group. Note that unimmunized animals had essentially no detectable IgE (<0.2 μg/ml). These results demonstrate that treatment with antigen and IL-2 reduces the
25   serum levels of allergic immunoglobulin IgE that plays a role in food allergy to eggs.

Example 4

This example shows that human T cells derived from a patient diagnosed with multiple sclerosis that are
30   specifically reactive with myelin basic protein can be deleted using the present invention. T cells were first stimulated for 48 hours, washed, and incubated with 300 units/ml IL-2 for 48 hours. The cells were then incubated on plastic dishes coated with 5/μg/ml of an antibody against the human T cell
35   receptor (TCR)/CD3 complex (MAb 64.1, which had been shown to potently activate T cells) for an additional 48 hours in the continued presence of 300 units/ml of IL-2. Flow cytometry analysis presented in Figure 20 shows that the samples of

cells carried in IL-2 and not rechallenged through the TCR/CD3 complex (none) contained 25790 cells/unit volume whereas cells that had been restimulated had 2974 cells/unit volume. Thus, restimulation of human T cells exposed to high IL-2 caused a
5   greater than 88% deletion of these cells.

        The invention being thus described, it will be obvious that the same may be varied in many ways. Such
10   variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

42

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WHAT IS CLAIMED IS:

1.   A method for treating or preventing a disease in a human or animal caused by antigen-activated T cells, comprising inducing the death by apoptosis of a subpopulation of T lymphocytes that is capable of causing said disease to an extent greater than that of other T lymphocytes.

2.   The method of claim 1, wherein said apoptosis is achieved by a cycle comprising challenging via immunization said T cells with a substance selected from the group consisting of an antigen, a peptide, a protein, a polysaccharide, an organic molecule, and a nucleic acid, followed by increasing the amount of IL-2 in said human or animal when said T cells are expressing high levels of IL-2 receptor, so as to cause said T cells to undergo apoptosis upon re-immunization with said substance.

3.   The method of claim 2, wherein said antigen, peptide, or protein is selected from the group consisting of one leading to an autoimmune disease, an allergic or atopic disorder, and graft rejection.

4.   The method of claim 3, wherein said autoimmune disease is selected from the group consisting of multiple sclerosis, uveitis, arthritis, Type I insulin-dependent diabetes, Hashimoto's thyroiditis, Grave's thyroiditis, and autoimmune myocarditis.

5.   The method of claim 3, wherein said allergic disorder is selected from the group consisting of hay fever, extrinsic asthma, insect bite and sting allergies, and food or drug allergies.

6.   The method of claim 2, wherein said challenging via immunization is conducted by administering said antigenic peptide or protein at a dose effective to cause said T cells to express high affinity IL-2 receptors and/or to produce and secrete IL-2.

7.   The method of claim 2, wherein said challenging
via immunization is conducted by administering said antigenic
peptide or protein at a dose between about 10 to about 1000
µg.

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8.   The method of claim 2, wherein said challenging
via immunization is conducted by administering said antigenic
peptide or protein orally or intramuscularly.

10   9.   The method of claim 3, wherein said antigenic
peptide or protein is selected from the group consisting of
myelin basic protein residue 84-102, myelin basic protein
residue 143-168, human S antigen, type II collagen,
thyroglobulin, Amb a V, Amb t V, an antigen inciting hay
15   fever, an antigen derived from insect venom, an antigen
derived from insect saliva, a food antigen, a drug antigen,
and a donor class I major histocom-patibility complex antigen.

10.   The method of claim 9, wherein said antigen
20   derived from insect venom is antigen V of hornet venom.

11.   The method of claim 9, wherein said food
antigen is codfish allergen M.

25   12.   The method of claim 2, wherein said challenging
via immunization is followed by a period of time between about
12 to about 72 hours before administering an additional high
dose of IL-2.

30   13.   The method of claim 2, wherein a high dose of
IL-2 is administered intravenously, either as a continuous
infusion or as frequent bolus doses.

14.   The method of claim 2, wherein a high dose of
35   IL-2 is in the range between about 300 to about 3,000
units/kg/hour continuous infusion, or from about 10^4 to about
10^6 units/kg intravenous bolus.

15. The method of claim 13, wherein said continuous infusion is conducted for a period of time between about 48 to about 72 hours.

16. The method of claim 2, wherein said cycle is repeated up to an endpoint selected from the group consisting of elimination of *in vitro* reactivity to said antigenic peptide or protein, amelioration of clinical symptoms, decreased allergic skin test, reduction in serum IgE, and toxicity.

17. The method of claim 9, wherein said donor class I major histocompatibility complex antigen is administered in the form of whole blood, packed cell equivalent, or a washed packed cell transfusion.

18. The method of claim 17, wherein said whole blood is administered in a dose of about 50 to about 200 ml.

19. The method of claim 18, wherein the total amount of whole blood administered is determined by the fluid tolerance of end-stage renal disease in the recipient.

20. The method of claim 19, wherein said cycle is repeated up to an endpoint selected from the group consisting of a diminished requirement for general immunosuppressive medications, graft survival, and adequate function of said graft.

21. The method of claim 2, wherein said cycle comprises challenging via immunization said T cells by repeated administration of said substance without the subsequent exogenous administration of IL-2.

22. The method of claim 21, wherein said cycle of repeated administration is performed at a short time interval.

23. The method of claim 22, wherein said time
interval is in the range of from about one to about five days.

24. The method of claim 22, wherein said time
interval is in the range of from about one to about three
days.